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CHEMICAL EXAMINATION
OF
CALABAR BEANS

BY

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THE WELLCOME CHEMICAL RESEARCH LABORATORIES

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CCXLII.—*Chemical Examination of Calabar Beans.*

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THE so-called Calabar beans, the ripe seed of *Physostigma venenosum*, Balfour, have previously been the subject of several investigations, and their most important constituent—the alkaloid physostigmine or eserine—has long been recognised by most of the national Pharmacopœias on account of its valuable medicinal properties.

The first chemical examination of Calabar beans was conducted by Jobst and Hesse (*Annalen*, 1864, 129, 115), who ascertained that their poisonous action is due to an alkaloid, which they designated physostigmine. This substance was obtained by them only as an amorphous, varnish-like mass, but a year later Vée (*Jahresber.*, 1865, 456) succeeded in isolating the alkaloid in a crystalline state. The last-mentioned investigator found that the alkaloid melted at 69°, and proposed for the crystalline base the name eserine. Jobst and Hesse, in a later investigation (*Annalen*, 1867, 141, 913), assigned to physostigmine the formula $C_{15}H_{21}O_2N_3$, but still expressed doubt regarding its crystalline character, although it is now known that under suitable conditions the alkaloid, as stated by Vée (*loc. cit.*), can be obtained in the crystalline form.

In the year 1876 Harnack and Witkowski (*Arch. experim. Path.*, 1876, 401) indicated the presence of a second alkaloid in Calabar beans, for which they proposed the name calabarine. This substance was very indefinite in character, being chiefly distinguished by its tetanus-like effects on the living organism. Subsequent investigations, notably that of Ehrenberg (*Verh. Ges. Deut. Naturf. Aertzte*, 1893, II, 102), have shown that calabarine was in all probability a product of decomposition, and could not have pre-existed in the Calabar bean. It is evident, however, that besides physostigmine a very small proportion of other alkaloids is contained in the Calabar bean; thus, in the year 1888, a crystalline base, differing from physostigmine, was obtained by Böhringer & Söhne (*Pharm. Post*, 1888, 21, 663), and termed by them eseridine. This base was stated to melt at 132°, to be much less poisonous than physostigmine, and on heating with dilute mineral acids to become converted into the latter alkaloid. Eseridine has been further examined by Eber (*Pharm. Zeit.*, 1892, 37, 483), who assigned to it the formula $C_{15}H_{23}O_3N_3$, and it is thus seen to differ from physostigmine only in the elements of one molecule of water.

Ehrenberg (*loc. cit.*) succeeded in isolating a third alkaloid from the Calabar bean. This substance, which is present in extremely

small proportions, was named eseramine, and the empirical formula $C_{16}H_{25}O_3N_4$ assigned to it. It was described as a substance very sparingly soluble in ether and melting at 238—240°. Still another alkaloid of Calabar bean has more recently been described by Ogui (*Apoth. Zeit.*, 1904, **19**, 891). This baso is stated to be isomeric with physostigmine, and therefore designated *isophysostigmine*. The only information concerning it appears to be the fact that it is insoluble in ether, and yields a sulphate melting at 200—202°.

Little is known regarding the constitution of physostigmine, and practically nothing of the alkaloids associated with it. It therefore seemed desirable to undertake some experiments for the purpose of throwing further light on the chemical constitution of these bases. To achieve this object a large quantity of Calabar beans was obtained and worked up for the isolation of the alkaloids. At the same time, the opportunity was taken to examine more completely the other constituents of the bean. The experiments undertaken in order to elucidate the constitution of physostigmine are reserved for a future communication.

EXPERIMENTAL.

A representative sample of the Calabar beans employed in this investigation, when assayed by the method of the United States Pharmacopœia, yielded 0.091 per cent. of alkaloid. The method referred to, however, was found to give results which were much too low, since the amount of physostigmine isolated when working on the large scale, as described below, was equivalent to 0.179 per cent. of the material employed. The low result by the above method of assay has been ascertained to be due to the fact that three extractions with ether (as required by the Pharmacopœia) are quite insufficient to remove the alkaloid completely from a solution which has been rendered alkaline with sodium hydrogen carbonate.

With the object of testing for the presence of an enzyme, 500 grams of powdered material were mixed with water and kept at the ordinary temperature for forty-eight hours. The liquid was then separated from the mass by filtration under pressure, and alcohol added to the filtrate. A caseous, white precipitate was thus produced, which was collected and dried in a vacuum desiccator over sulphuric acid, when it amounted to 14.5 grams. This substance, which gave the biuret reaction, slowly hydrolysed amygdalin, thus indicating the presence of an enzyme.

A further portion (50 grams) of the powdered bean was successively extracted in a Soxhlet apparatus with various solvents, when

the following amounts of extract, dried at 100° , were obtained:

Petroleum (b. p. $35-50^{\circ}$) extracted	0.38	grams =	0.76	per cent.
Ether	0.08	,, =	0.16	,,
Chloroform	0.18	,, =	0.36	,,
Ethyl acetate	0.13	,, =	0.26	,,
Alcohol	2.32	,, =	4.64	,,
Total.....	3.09	,,	6.18	,,

For the purpose of a complete examination of the constituents of the bean 122.7 kilograms of finely powdered material were extracted by continuous percolation with hot alcohol. After removal of the greater part of the alcohol there remained a reddish-brown extract, amounting to $10\frac{1}{2}$ kilograms. This extract, in convenient portions, was mixed with water, and the mixture distilled in a current of steam. The distillate, which contained a very small quantity of essential oil, was extracted with ether, the ethereal solution being washed, dried, and the ether removed. A pale yellow oil possessing a garlic-like odour and amounting to 1.5 grams was obtained.

After the above operation there remained in the steam distillation flask a large quantity of fatty oil floating on the surface of a reddish-brown aqueous liquid, which contained a brown, resinous powder in suspension. The fatty oil was separated, washed well with water, and set aside for subsequent examination, whilst the brown resin was collected and washed with water, the washings being added to the main portion of the aqueous liquid.

Isolation of Physostigmine, $C_{15}H_{21}O_2N_3$.

A small portion of the aqueous liquid was, in the first place, extracted successively with ether and amyl alcohol, but these solvents removed only small quantities of gummy material. The whole of the aqueous liquid was then rendered alkaline with an excess of sodium carbonate, and repeatedly extracted with ether until no further alkaloidal substance was removed by this treatment. The ethereal extracts were united, concentrated to a convenient volume, and then carefully shaken with successive portions of 5 per cent. sulphuric acid until the extract became just acid in reaction. The neutral solution of the sulphate of the alkaloid was then treated with an excess of a saturated solution of sodium salicylate, when the salicylate of the alkaloid was precipitated as an almost colourless, crystalline powder. This was collected, washed well with water, and dried in a vacuum desiccator over sulphuric acid. A further quantity of the salicylate of the alkaloid was obtained from the filtrate by rendering it alkaline with sodium carbonate, extract-

ing with ether, and subjecting the ethereal solution to the same process as that described above. The total crude salicylate thus obtained amounted to 330 grams, representing 219.7 grams of physostigmine, which is equivalent to 0.179 per cent. of the material employed in the investigation. In order to ascertain whether this crude salicylate was homogeneous or not, a considerable portion of the compound was subjected to systematic fractional crystallisation from alcohol. The fractions were found to be uniform in character, crystallising in stout, colourless prisms, melting at 180—181°, and were therefore homogeneous. (Found, C=64.1; H=6.6; N=10.2. Calc., C=63.9; H=6.5; N=10.2 per cent.)

It is evident that the above compound consisted of physostigmine salicylate. The free base was obtained from the latter by agitation with aqueous sodium carbonate in the presence of ether. The ethereal solution of physostigmine thus obtained was dried, and the solvent removed. The pale brown, viscid residue was dissolved in benzene, and the solution allowed to evaporate slowly at the ordinary temperature in a vacuum desiccator over sulphuric acid. After some time there was deposited a mass of crystals, which was collected and recrystallised several times from a mixture of benzene and petroleum. The physostigmine separated from this solvent in stout prisms, which melted at 86—87°. (Found, C=65.1; H=7.9. Calc., C=65.5; H=7.6 per cent.)

0.2659, made up to 20 c.c. with chloroform, gave $\alpha_D - 2^\circ 1'$ in a 2-dcm. tube, whence $[\alpha]_D - 75.8^\circ$.

The above specimen of physostigmine does not agree in melting point with the physostigmine obtained by Pictet and Polonowsky (*Bull. Soc., chim.*, 1893, [iii], 9, 1008), who state that the alkaloid melts at 105—106°. A commercial sample of physostigmine was therefore procured, and this also melted at 105—106°. It was then found that the alkaloid is dimorphous, since the modification melting at 86—87° could be readily converted into the substance melting at 105—106° by recrystallisation in the presence of a crystal of the latter. The optical rotation of the compound melting at 105—106° was determined, and found to be identical with that of the modification melting at 86—87°, as recorded above:

0.3062, made up to 20 c.c. with chloroform, gave $\alpha_D - 2^\circ 19'$ in a 2-dcm. tube, whence $[\alpha]_D - 75.8^\circ$.

Pictet and Polonowsky (*loc. cit.*) ascribe to physostigmine an optical rotation, $[\alpha]_D - 82^\circ$, which is somewhat higher than the results obtained above.

Physostigmine does not yield any definite aurichloride or platinochloride, but a well-defined *picrate* was obtained, which crystallises

from dilute alcohol in feathery, yellow needles, melting at 114° . It is only sparingly soluble in hot water, but readily so in alcohol.

Isolation of Eseramine.

The ethereal liquid which had been shaken with dilute sulphuric acid for the separation of the physostigmine as above described was washed with water, dried, and the solvent removed. A small amount (about 2 grams) of a viscid, brown residue was thus obtained, which gradually became solid. Since the solid was not completely soluble in ether, it was digested with a small quantity of the latter, the sparingly soluble portion collected, and then purified by crystallisation from alcohol, when it separated in small, colourless needles, melting and decomposing at 245° . This substance was very sparingly soluble in ether, chloroform, or benzene, but readily so in hot alcohol. It dissolved in strong mineral acids, and yielded a precipitate with Mayer's reagent. It appears from the above properties that the substance is identical with the eseramine of Ehrenberg (*loc. cit.*), but the amount isolated (0.1 gram) did not admit of further examination.

Isolation of a New Alkaloid, Physovenine, $C_{14}H_{18}O_3N_2$.

The ethereal liquid from which the above-mentioned eseramine had been removed, yielded on evaporation a brown, oily residue, which slowly crystallised. The crystals were separated from a little adhering oil, and then crystallised, first from dilute alcohol, and finally from a mixture of benzene and light petroleum. The substance separated from the latter solvent in small, colourless prisms, melting at 123° . The yield of pure substance amounted to 0.6 gram:

0.1024 gave 0.2395 CO_2 and 0.0669 H_2O . $C=63.8$; $H=7.3$.

0.1165 „ 0.2726 CO_2 „ 0.0736 H_2O . $C=63.8$; $H=7.0$.

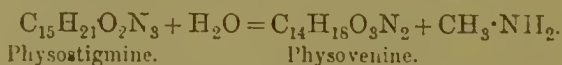
0.1817 „ 17.2 c.c. N_2 (moist) at 25° and 764 mm. $N=10.6$.

$C_{14}H_{18}O_3N_2$ requires $C=64.1$; $H=6.9$; $N=10.7$ per cent.

The above-described alkaloid is not identical with any of the bases previously isolated from Calabar beans, and since no compound of the formula $C_{14}H_{18}O_3N_2$ appears to have been hitherto described, it is proposed to designate the new alkaloid, *physovenine*.

Physovenine, $C_{14}H_{18}O_3N_2$, is very soluble in alcohol, benzene, or chloroform, moderately so in ether, but insoluble in light petroleum or water. It is only a weak base, dissolving in concentrated mineral acids, but being reprecipitated on the addition of water. When physovenine is treated with barium hydroxide, there is an immediate precipitation of barium carbonate, and the solution rapidly

assumes a deep red colour. This behaviour of physovenine is very similar to that of physostigmine, since the latter under the influence of alkalis (Ehrenberg, *loc. cit.*) loses carbon dioxide and methylamine, and becomes converted successively into eseroline and a red colouring matter, rubreserine. It appears probable from the properties and composition of physovenine that the latter is an intermediate product in the conversion of physostigmine into eseroline, as represented by the following equations:



Physostigmine.

Physovenine.



Eseroline.

Physovenine is, like physostigmine, very powerfully myotic; thus a single drop of a 0.1 per cent. solution of the alkaloid in dilute alcohol when introduced into the eye produced after an interval of a few minutes a powerful contraction of the pupil, which attained its maximum effect half an hour after the injection.

A portion of the aqueous liquid from which the alkaloids had been extracted as described above, was rendered slightly acid with acetic acid, and then concentrated to a small bulk. A large amount of a viscid syrup was thus obtained, which readily reduced Fehling's solution. It was digested with hot alcohol, the alcoholic liquid decanted, and ethyl acetate added to the latter. In this manner a considerable quantity of a sugar was obtained, which yielded a glucosazone, melting and decomposing at 205°.

The Fatty Oil.

The fatty oil which had been isolated as previously described amounted to 1650 grams. Since it was found to contain a small quantity of alkaloidal substance of weakly basic character, it was extracted with 40 per cent. sulphuric acid. The acid extracts were rendered alkaline and extracted with ether. In this manner a small amount (1.5 grams) of a brown, viscid liquid was obtained, which partly solidified after keeping some time. On examination, the crystalline substance was found to be identical with the alkaloid physovenine, $\text{C}_{14}\text{H}_{18}\text{O}_3\text{N}_2$, previously isolated from the aqueous liquid.

A convenient portion (200 grams) of the fatty oil was next hydrolysed by heating with an excess of potassium hydroxide in the presence of alcohol, when a considerable quantity of ammoniacal vapours was evolved. After this treatment the greater part of the alcohol was removed, water added, and the alkaline liquid repeatedly

extracted with ether. The pale yellow ethereal extract was washed, dried, and the ether removed, when 10 grams of a crystalline residue were obtained. This was dissolved in hot alcohol, from which it separated in colourless leaflets, melting at $132-133^{\circ}$. This substance gave the colour reactions of the phytosterols, and evidently belonged to that class of compounds. Windaus and Hauth (*Ber.*, 1906, **39**, 4378; 1907, **40**, 3681) have already shown that the phytosterol of Calabar bean is a mixture of two compounds, namely, stigmasterol, $C_{30}H_{48}O$, and sitosterol, $C_{27}H_{46}O$, which were separated by them after conversion into the bromoacetyl derivatives. In order to ascertain whether the above crude phytosterol, melting at $132-133^{\circ}$, consisted of such a mixture, the substance was acetylated, and the acetyl derivative treated with bromine in the presence of acetic acid. The sparingly soluble bromo-derivative which separated was purified by crystallisation from a mixture of chloroform and alcohol, and was obtained in colourless leaflets, melting and decomposing at $205-206^{\circ}$. (Found, Br=40.6. Calc., Br=40.7 per cent.) This substance is evidently identical with the tetrabromoacetylstigmasterol described by Windaus and Hauth (*loc. cit.*). When debrominated by means of zinc dust the above compound yielded acetylstigmasterol (m. p. 140°), and the latter on hydrolysis was converted into stigmasterol, melting at 169° .

The mother liquors from the bromination, treated in the same manner, yielded successively acetylsitosterol (m. p. 128°) and sitosterol, $C_{27}H_{46}O$, melting at $134-135^{\circ}$. (Found, C=83.7; H=12.1. Calc., C=83.9; H=11.9 per cent.) It is thus evident that the above crude phytosterol, melting at $132-133^{\circ}$, consists of a mixture of stigmasterol and sitosterol.

Isolation of Trifolanol, $C_{21}H_{34}O_2(OH)_2$.

The alkaline aqueous liquid from which the above-described crude phytosterol had been extracted by ether was acidified with dilute sulphuric acid, when a dark-coloured, semi-solid precipitate of fatty acids was produced. A portion of the precipitate was observed to be sparingly soluble in ether, and this was therefore separately collected, when it formed a dark-coloured solid amounting to 5 grams. This sparingly soluble substance was first digested with hot alcohol, which removed the greater part of the colouring matter, and then treated with boiling chloroform. The latter solvent extracted a small amount of a colourless solid, which was purified by crystallisation from dilute pyridine, when it separated in microscopic needles, melting and decomposing at 295° . When dissolved in chloroform and acetic anhydride and a drop of concentrated sulphuric acid

added, it gave a transient pink coloration, changing to blue, and then to green. (Found, $C=71.4$; $H=10.2$. Calc., $C=71.6$; $H=10.2$ per cent.)

The properties of the above compound, together with the result of its analysis, indicate that the substance is identical with trifolianol, $C_{21}H_{31}O_2(OH)_2$, which was first isolated from red clover flowers (Power and Salway, Trans., 1910, 97, 249). Its identity was confirmed by the formation of diacetyltrifolianol, which crystallised in flat, colourless needles, melting at $165-166^\circ$. (Found, $C=68.9$; $H=9.2$. Calc., $C=68.8$; $H=9.2$ per cent.):

0.2118, made up to 20 c.c. with chloroform, gave $\alpha_D = 0.031'$ in a 2-dcm. tube, whence $[\alpha]_D = 24.4^\circ$.

Isolation of a New Dihydric Alcohol, Calabarol, $C_{23}H_{31}O_2(OH)_2$.

The sparingly soluble substance which had been digested with chloroform for the removal of the trifolianol, as above described, was crystallised from dilute pyridine, when it separated in minute crystals, melting and decomposing at $290-300^\circ$. It was found, however, that this substance still contained a considerable proportion of trifolianol. A separation was effected by converting the substance into its benzoyl derivative and subjecting the latter to a systematic fractional crystallisation from a mixture of chloroform and alcohol. In this manner two benzoyl compounds were isolated, melting at $165-170^\circ$ and $195-196^\circ$ respectively. The former was found on analysis to be *dibenzoyltrifolianol*:

0.1109 gave 0.3052 CO_2 and 0.0822 H_2O . $C=75.1$; $H=8.2$.

$C_{35}H_{44}O_6$ requires $C=75.0$; $H=7.9$ per cent.

The benzoyl derivative melting at $195-196^\circ$ was analysed with the following results:

0.1173 gave 0.3268 CO_2 and 0.0810 H_2O . $C=76.0$; $H=7.7$.

0.1088 „ 0.3036 CO_2 „ 0.0768 H_2O . $C=76.1$; $H=7.8$.

$C_{37}H_{44}O_6$ requires $C=76.0$; $H=7.5$ per cent.

0.5894, made up to 20 c.c. with chloroform, gave $\alpha_D + 0.052'$ in a 2-dcm. tube, whence $[\alpha]_D + 14.7^\circ$.

It would appear from the above analysis that the benzoyl compound melting at $195-196^\circ$ is derived from a dihydric alcohol, $C_{23}H_{36}O_4$. In order to isolate this compound in a state of purity a quantity of the original mixture of trifolianol and the new alcohol was heated with equal quantities of chloroform and alcohol, and the undissolved substance collected. On repeating the process several times a homogeneous compound was obtained, which melted at 245° . This was recrystallised from dilute pyridine, when it separated in colourless, microscopic needles:

0.0504 gave 0.1351 CO_2 and 0.0460 H_2O . $\text{C}=73.1$; $\text{H}=10.1$.

$\text{C}_{23}\text{H}_{38}\text{O}_4$ requires $\text{C}=73.4$; $\text{H}=9.6$ per cent.

The above substance is thus seen to possess the formula $\text{C}_{23}\text{H}_{38}\text{O}_4$. As it is a new compound it is proposed to designate it *calabarol*, with reference to the source from which it has been obtained.

Calabarol is very sparingly soluble in the usual organic solvents, but dissolves readily in pyridine. When dissolved in acetic anhydride and chloroform and a drop of concentrated sulphuric acid added, a pink coloration is produced, which rapidly changes to blue and then to green. Calabarol yields a *dibenzoyl* derivative, $\text{C}_{23}\text{H}_{34}\text{O}_4(\text{CO}\cdot\text{C}_6\text{H}_5)_2$, which crystallises from a mixture of chloroform and alcohol in well-formed, colourless needles, melting at $195\text{--}196^\circ$. It is readily soluble in ethyl acetate or chloroform, but very sparingly so in alcohol.

The properties of calabarol indicate that it is closely allied to a number of dihydric alcohols, which have been isolated in these laboratories, and which fall into two groups possessing the general formulæ $\text{C}_n\text{H}_{2n-6}\text{O}_4$ and $\text{C}_n\text{H}_{2n-8}\text{O}_4$ respectively. Calabarol, however, appears to belong to a new series of dihydric alcohols, which may be represented by the general formula $\text{C}_n\text{H}_{2n-10}\text{O}_4$.

Examination of the Fatty Acids.

The ethereal solution of fatty acids from which trifoliantol and calabarol had been removed as described above, was washed, dried, and the ether removed. The residue was dissolved in hot alcohol, when a substance separated, which, after several crystallisations from alcohol, melted at $74\text{--}76^\circ$:

0.3073 required, for neutralisation, 8.85 c.c. $N/10\text{-KOH}$. Neutralisation value = 161.5.

$\text{C}_{22}\text{H}_{44}\text{O}_2$ requires neutralisation value = 165.0.

It appears from this result that the substance is behenic acid (m. p. $80\text{--}82^\circ$), although the melting point is somewhat low for the latter compound. In order, therefore, to effect a further purification, the substance was esterified, and the ethyl ester thus produced then distilled under diminished pressure. The first portion of the distillate having been discarded, the remainder was hydrolysed, when it yielded a fatty acid, melting at 77.5° . (Found, $\text{C}=77.6$; $\text{H}=13.0$. Calc., $\text{C}=77.7$; $\text{H}=12.9$ per cent.):

0.1775 required for neutralisation 5.2 c.c. $N/10\text{-KOH}$. Neutralisation value = 164.3.

$\text{C}_{22}\text{H}_{44}\text{O}_2$ requires neutralisation value = 165.0.

The substance was thus definitely identified as behenic acid

The remaining portion of the fatty acids was converted into the lead salt, and the latter treated with ether in the usual manner for the separation of the saturated from the unsaturated acids. The portion of lead salt insoluble in ether was collected, and the fatty acids regenerated and crystallised from alcohol. The first crystalline deposits consisted of behenic acid, melting at 77° . The subsequent deposits melted at about 55 — 60° , and were evidently mixtures. In order to ascertain the constituents of this product, it was dissolved in alcohol and fractionally precipitated by the successive addition of small portions of a concentrated solution of barium acetate. In this manner four fractions were obtained, from each of which the fatty acid was regenerated, then crystallised once from alcohol, and titrated with $N/10$ -potassium hydroxide. The neutralisation values of the several fractions were 202.5, 205.9, 207.0, and 209.5, whilst their melting points were 55° , 59° , 59° , and 60° respectively. These results indicated the presence of palmitic and stearic acids.

The unsaturated acids obtained from that portion of the lead salt which was soluble in ether amounted to 80 grams. When distilled under diminished pressure a yellow oil which passed over at 215 — $240^{\circ}/15$ mm. was obtained. An analysis and a determination of the constants led to the following results:

0.1064 gave 0.3004 CO_2 and 0.1128 H_2O . $\text{C}=77.0$; $\text{H}=11.8$.

Neutralisation value = 199.6; Iodine value = 139.

$\text{C}_{18}\text{H}_{34}\text{O}_2$ requires $\text{C}=76.6$; $\text{H}=12.1$ per cent. Neutralisation value = 198.9; Iodine value = 90.1.

$\text{C}_{18}\text{H}_{32}\text{O}_2$ requires $\text{C}=77.1$; $\text{H}=11.4$ per cent. Neutralisation value = 200.4; Iodine value = 181.4.

It is thus evident that the liquid acids consisted of a mixture of oleic and linolic acids.

Examination of the Resins.

The resin which had been separated from the aqueous liquid in the manner previously described, consisted for the most part of a brown powder, which was very sparingly soluble in the usual organic solvents. This product was mixed intimately with purified sawdust, the mixture dried, and then extracted in a Soxhlet apparatus successively with petroleum, ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin.

This consisted of a brown, fatty oil, amounting to 65 grams. In order to ascertain whether the oil contained any alkaloid, it was dissolved in ether, and the ethereal solution shaken with dilute

sulphuric acid. The acid extracts yielded, however, only a very small quantity of a brown oil, which apparently consisted of physostigmine.

The ethereal solution which had been shaken with dilute sulphuric acid was washed, dried, and the solvent removed. The residue was then hydrolysed, and the products of hydrolysis examined in a manner similar to that previously described in connexion with the fatty oil of Calabar bean. The following constituents were found to be present: stigmasterol, sitosterol, trifolianol, calabarol, and behenic, palmitic, stearic, oleic, and linolic acids.

Ether, Chloroform, and Ethyl Acetate Extracts of the Resin.

These extracts were dark brown, gummy solids, amounting to 5, 17.5, and 11 grams respectively. They were separately examined, but nothing of a definite nature was isolated from them.

Alcoholic Extract of the Resin.

This was a dark brown syrup amounting to 41 grams. It was dissolved in alcohol, and the solution heated for some time with dilute sulphuric acid. After removal of the alcohol in a current of steam, there remained a black, resinous mass and a deeply coloured aqueous liquid. The former was collected and examined, but yielded nothing definite. Since a small portion of the aqueous liquid gave a precipitate with Mayer's reagent, the whole was rendered alkaline with sodium carbonate, and the alkaline mixture extracted repeatedly with ether. The ethereal extracts were united, and agitated with small portions of dilute sulphuric acid until the liquid was just acid in reaction. The extracts thus obtained when treated with a concentrated solution of sodium salicylate, yielded 3 grams of a crystalline solid, which was found to be identical with physostigmine salicylate, melting at 180—181°.

The alkaloid isolated as above described could not have existed as such in the alcohol extract of the resin, but was evidently present in some form of combination.

Summary.

The results of this investigation have shown that the Calabar bean (*Physostigma venenosum*, Balfour) contains, in addition to some essential oil, resin, and other amorphous substances, the following compounds:

Physostigmine, $C_{15}H_{21}O_2N_3$.—This alkaloid was found to be dimorphous, since two interconvertible modifications were obtained, melting at 86—87° and 105—106° respectively. The amount of

alkaloid isolated was equivalent to 0.179 per cent. of the Calabar beans employed.

Physovenine, $C_{14}H_{18}O_3N_2$, a new alkaloid, melting at 123° . Like physostigmine, it produces a powerful myotic effect on the pupil of the eye.

Eseramine, an alkaloid melting at 245° .

Calabarol, $C_{23}H_{38}O_4$, a new dihydric alcohol (m. p. 245°), which yields a *dibenzoyl* derivative, melting at 195 — 196° .

Trifolianol, $C_{21}H_{36}O_4$, a dihydric alcohol previously isolated from red clover flowers.

Stigmasterol, $C_{30}H_{48}O$, and sitosterol, $C_{27}H_{46}O$.

Glycerides of behenic, stearic, palmitic, oleic, and linolic acids.

A sugar yielding *d*-phenylglucosazone (m. p. 205°).

In the course of the present investigation no evidence has been obtained of the presence in Calabar beans of the alkaloid designated by Ogui (*loc. cit.*) as "*isophysostigmine*," or of the "*eseridine*" of Böhringer & Söhne (*loc. cit.*).

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